P.C. 2108.91

PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of)
)
LAWRENCE A. JOHNSON)
)
Method to Preselect the)
Sex of Offspring)

Group Art Unit 188
Examiners J. Witz &
D. W. Robinson

DECLARATION UNDER CFR 1.132

Lawrence A. Johnson deposes and states:

1. That he is the applicant of the above-identified application;

2. That he conducted the following study designed to compare the absorbance of fluorescent dye by mammalian sperm incubated at room temperature (approximately 22° C) with the absorbance of fluorescent dye by mammalian sperm cells incubated at 35° C:

> Cattle semen was collected by standard methods and was allowed to equilibrate to room temperature. The semen was diluted with Tris buffer, pH 6.9, to a concentration of 10 x 10^6 per ml. The semen was then divided into 1-milliliter samples in tubes, and bisbenzimide Hoechst 33342 fluorescent stain was added at a concentration of 5 μg/ml to each tube. The tubes of semen and stain were either allowed to incubate at room temperature or were heated over a water bath to 35° C for various periods of time. At the conclusion of each incubation period, the samples were put through a flow cytometer where the fluorescently labeled cells were excited in the ultraviolet lines (UV; 361 and 364 nm) lines of a 5-watt 90-5 Innova Argon-ion laser

operating at 200 mW. Data were collected as 256-channel histograms indicating the number of sperm versus the intensity of fluorescence. The results are shown in Figures A-G of Exhibit A, submitted herewith;

3. That the sperm incubated at 35° for periods of 15 min, 1 hr, 3 hrs and 5 hrs, were analyzed For DNA Fluorescence ~~tested~~ on a modified EPICS V flow cytometer/cell sorter using a sheath fluid comprising 10 mM phosphate-buffered saline containing 0.1% bovine serum albumin wherein the modification to the cell sorter consisted of a beveled sample insertion tube and a forward (0°) fluorescence detector;

4. That it is apparent from Figure A of Exhibit A that a large portion of the sperm population incubated for 15 min at room temperature exhibited a low level of fluorescence (peak on left), and a minor portion of the sperm exhibited a high level of fluorescence (peak on right);

5. That it is apparent from Figure E of Exhibit A that virtually all sperm incubated at 35° C for 15 min exhibited a high level of fluorescence;

6. That it is apparent from Figure B of Exhibit A that a major portion of the sperm population incubated for 1 hour at room temperature exhibited a low to moderate level of fluorescence (peak on left), and a minor portion of the sperm exhibited a high level of fluorescence (peak on right);

7. That it is apparent from Figure F of Exhibit A that virtually all sperm incubated at 35° C for 1 hr exhibited a high level of fluorescence and that the histogram of such is characterized by two peaks indicative of two subpopulations of sperm exhibiting high fluorescence;

8. That it is apparent from Figure C of Exhibit A that a minor portion of the sperm incubated at room temperature for 3 hr exhibited a moderate level of fluorescence and a major portion of the sperm exhibited a high level of fluorescence;

9. That it is apparent from Figure D of Exhibit A that virtually all the sperm incubated at room temperature for 5 hrs exhibited a high level of fluorescence;

10. That in Figure G of Exhibit A, the peak on the left represents the subpopulation of male-producing sperm (Y-bearing), and the peak on the right represents the female-producing sperm (X-bearing);

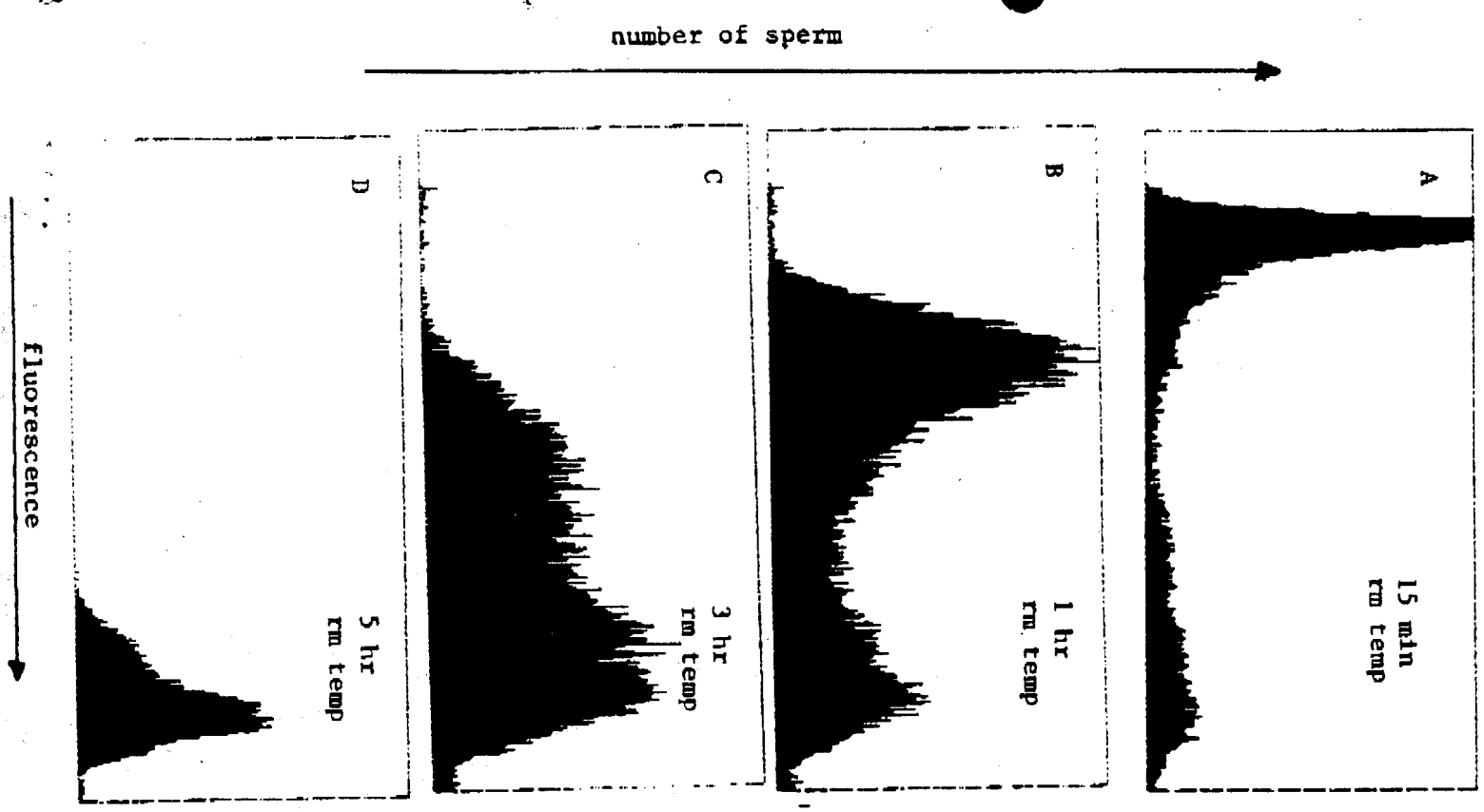
11. That based upon his knowledge of sperm staining, he would interpret the peaks representing high levels of fluorescence (right half of Figures A and B) to be indicative of dead sperm cells because of the porosity of the dead sperm membrane to the stain; that Figures C and D show a combination of dead and live sperm, since live sperm will take up the stain at room temperature if given enough time (5 hrs); that a higher percentage of sperm in C and D are dead because of the long time period required to take up the sperm; that the peaks of Figures E and F will consist predominantly of live sperm with whtever dead sperm that were initially present also being stained; live sperm are dependent on the elevated incubation temperature (35° C) to take up the stain in the short time period; and

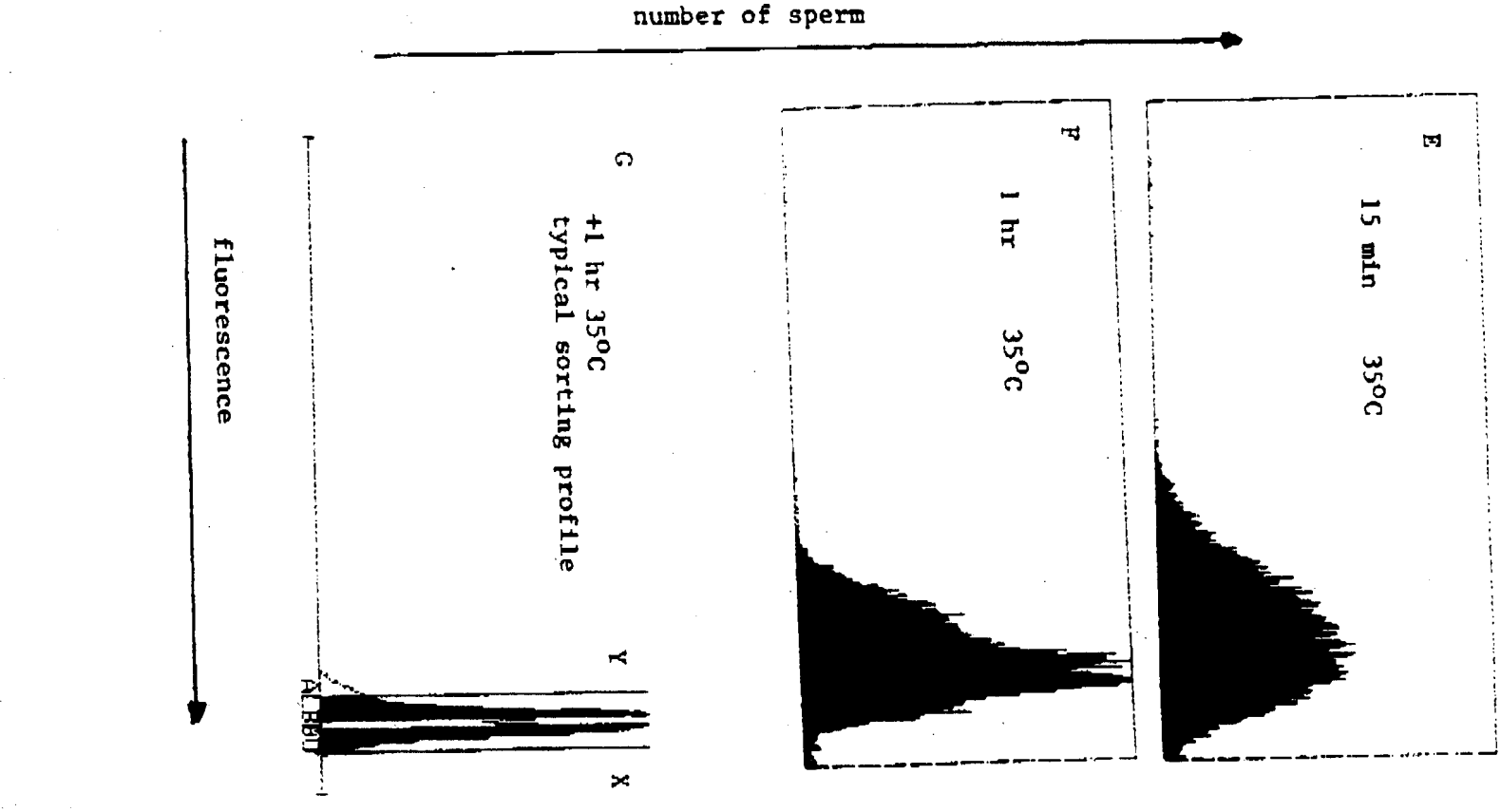
Serial No. 07/349,669

12. That all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed May 16, 1991. Lawrence A. Johnson

Enclosure:
Exhibit A





In re LAWRENCE A. JOHNSON
Serial No. 07/..... [P.C. 2108.91]
Johnson Declaration Under 37 CFR 1.132